PCL/graphene 3D scaffold fabrication and in vitro analysis using a solvent casting and particulate leaching assembly process

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1. ABSTRACT

Introduction

The surface characteristics of the scaffold provide a surface that is suitable for bone cell growth. Consequently, cells require materials that support these circumstances. The combination of two or more materials (composite) will provide more benefits by correcting for each other's weaknesses. Hence, the purpose of this study was to investigate the ability of PCL/graphene to enhance the osteoinductive mechanism.

Materials and Method

The PCL/graphene scaffold was developed utilizing a solvent casting and particulate leaching method and cultured with MG63 cell-like osteoblast cell lines at 0.5, 1.5, and 2.5 wt% of graphene. The study evaluated the porosity, wettability, scaffold morphology, chemical, mechanical, and chemoattractant characteristic, biocompatibility cells, and biodegradation

Results and Discussion

Graphene enhanced the surface properties of the scaffold that make it suitable for cell growth, whereas 2.5 wt% of graphene exhibited better than other concentrations.

Conclusion

This finding suggests that PCL/graphene composites have potential applications in bone engineering.

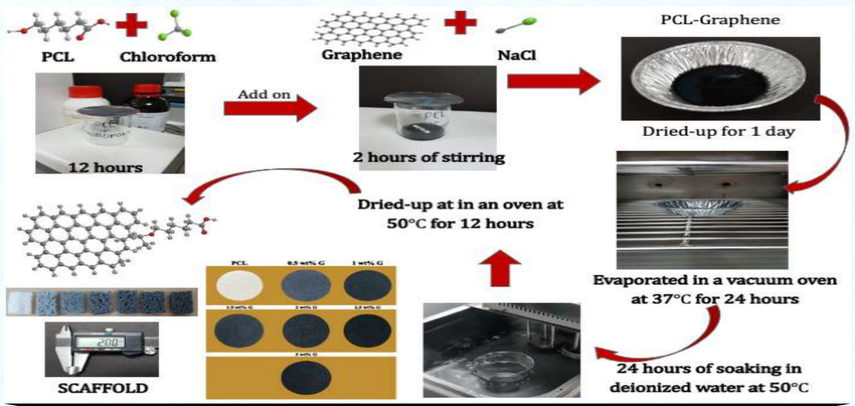
Keywords: Solvent casting/particulate leaching method, Surface characteristic, Chemoattractant, Biocompatibility, Biodegradation.

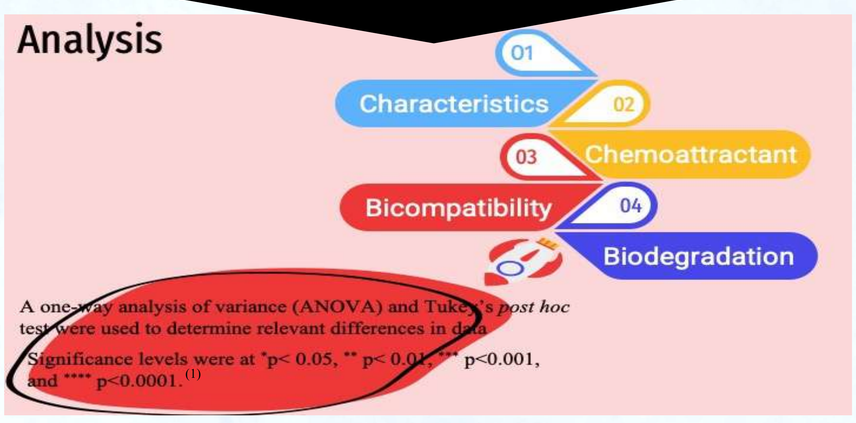
2. INTRODUCTION

Complex biological systems require a 3D framework to maintain their functional integrity, thus the fields of cell biology and material science must be integrated to create biomaterials that enhance osteoinductive and osteoconductive. Several methods and technologies have been develop for making 3D scaffolds, such as a solvent casting and particulate leaching method. The advantages of this method are (1) the process is simple and easy to carry out without the need for specific and expensive equipment; (2) it allows to control the final porosity, pore size, and interconnectivity; (3) the crystallinity of the porous can be controlled closely; (4) improves the mechanical and water barrier properties.

Polycaprolactone (PCL) has received a lot of attention in bone tissue engineering, However, the lack of mechanical qualities of PCL scaffolds limits their applicability. As a result, combining with graphene can improve it. Several studies revealed that graphene has a high specific surface area, chemical functionalization, and excellent protein adhesion than could modify extracellular environment to enhance osteoinductive and osteoconductive process. Therefore the scaffold should have several good criteria that suitable for bone cell, such as has chemoattractant, biocompatibility, and biodegradable ability. (1)

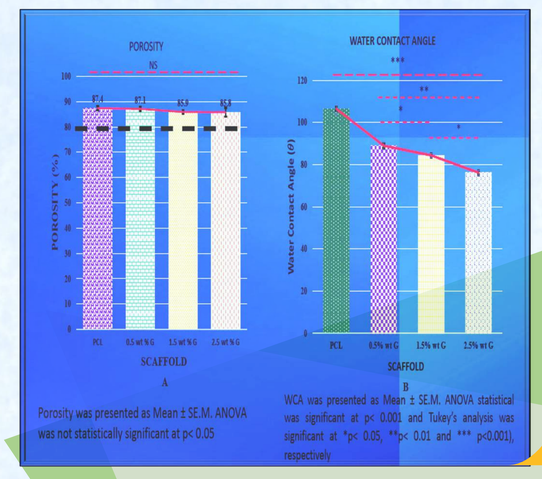
3. METHODS





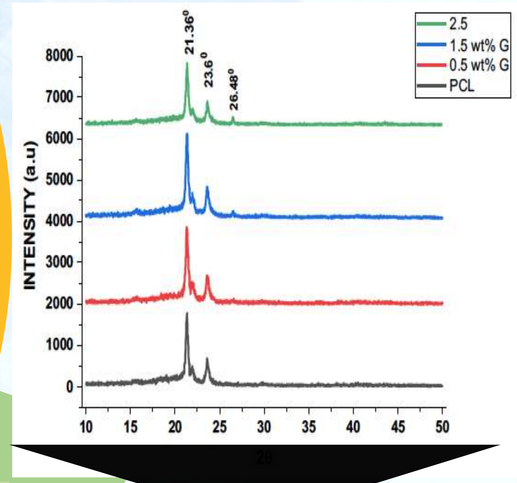
4. RESULTS AND DISCUSSION

A. CHARACTERISTICS



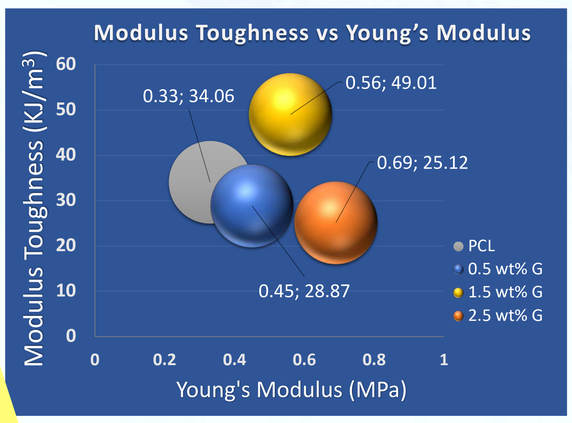
Porosity should increase both the scaffold's surface and within area, which can enhance the rate of water enters to the scaffold. The study revealed, the average porosity of PCL/graphene scaffolds were above 85%, which were ideal compared to cancellous bone porosity (79.3%). This circumstance might have altered the level of fluid shear on bone cells to adhere and proliferate on the scaffold.

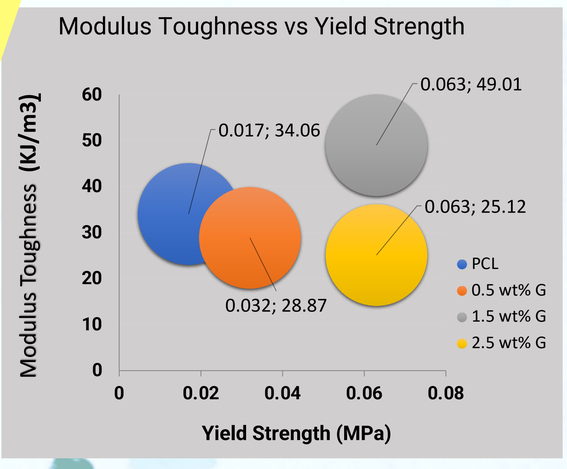
Not only porosity, but the hydrophilicity of the surface scaffolds is also well-known as a key factor in governing cell response. The ideal surface for cell attachment was a surface that had in the range 70-75°, because it leads to an increase in protein adsorption, which, in turn, plays an essential part in improving cell attachment by encouraging the formation of focal adhesions. (2)



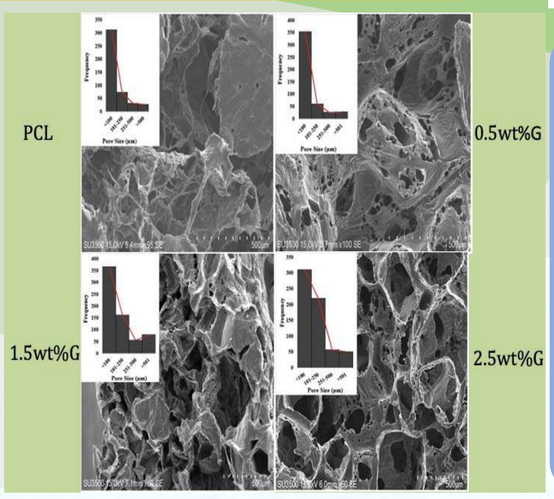
Two major peaks were found at values of 2θ = 21.36° and 23.6° in the diffraction pattern of PCL. The addition of graphene did not have a substantial impact on the value of 2θ = 21.36°, with the exception of a slight decrease in the peak at 2θ = 23.6°. On the other hand, the peak at 2θ = 26.48° improved as the concentration of graphene increased. Increasing the amount of graphene resulted in an increased concentration of functionalized oxygen and an enhanced capacity of graphene to disperse in water or cell culture media, both of which have been shown to increase cell viability.(4)

Graphene has high strength and stiffness but relatively low toughness. It is a type of brittle material, yet it can aid accelerate the degradation process of composites, especially polymers with low degradability. According to the data, 1.5 has higher modulus toughness than 2.5 wt% G, although 2.5 wt% G has a high Young's modulus. Both 1.5 and 2.5 wt% G were stronger, stiffer, and modulus toughness than PCL. Modulus toughness indicates a material's resistance to degradation, while strength displays its resistance to stress. Despite its stiffness, it enhanced the surface area of the scaffold and facilitated cell adhesion. Thus, 2.5 wt% G has better strength, cell adhesion, and degradation compared to others.[5]



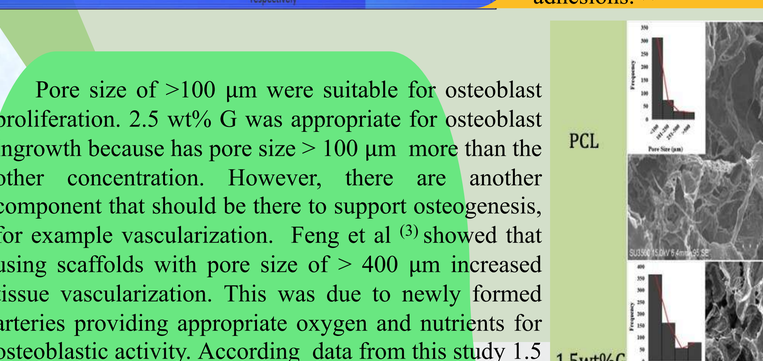


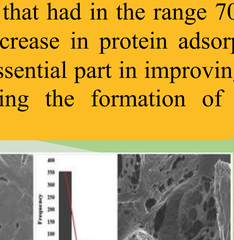
Pore size of >100 µm were suitable for osteoblast proliferation. 2.5 wt% G was appropriate for osteoblast ingrowth because has pore size > 100 µm more than the other concentration. However, there are another component that should be there to support osteogenesis, for example vascularization. Feng et al (3) showed that using scaffolds with pore size of > 400 µm increased tissue vascularization. This was due to newly formed arteries providing appropriate oxygen and nutrients for osteoblastic activity. According data from this study 1.5 and 2.5 wt% G have pore size of 251-500 µm and >501 µm more than others. Hence, PCL/graphene with high concentration of graphene appropriate for osteogenesis.



B. CHEMOATTRACTAN

Table: Cell migration at 24 and 48 hours in MG-63 scratch assay



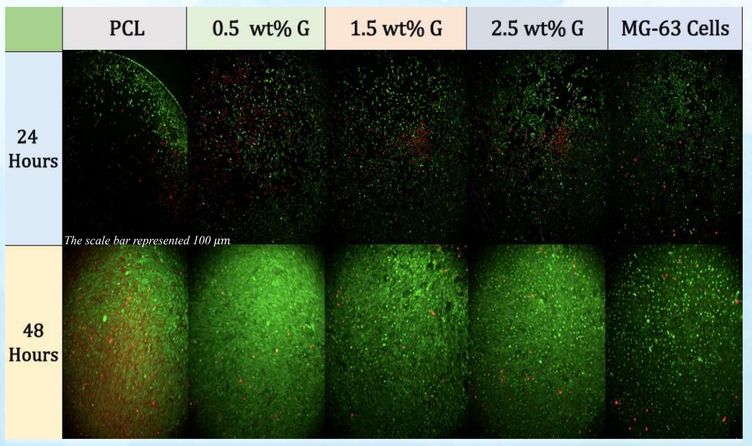


The data revealed that PCL/graphene scaffolds with various graphene concentrations had greater viability than PCL scaffolds ($p \leq 0.001$) with 2.5 wt% graphene scaffolds exhibiting a smaller density of dead cells than the others ($p \leq 0.05$) and up to 5-fold fewer compared to PCL scaffolds ($p \leq 0.001$) after 24 hours of degradation.

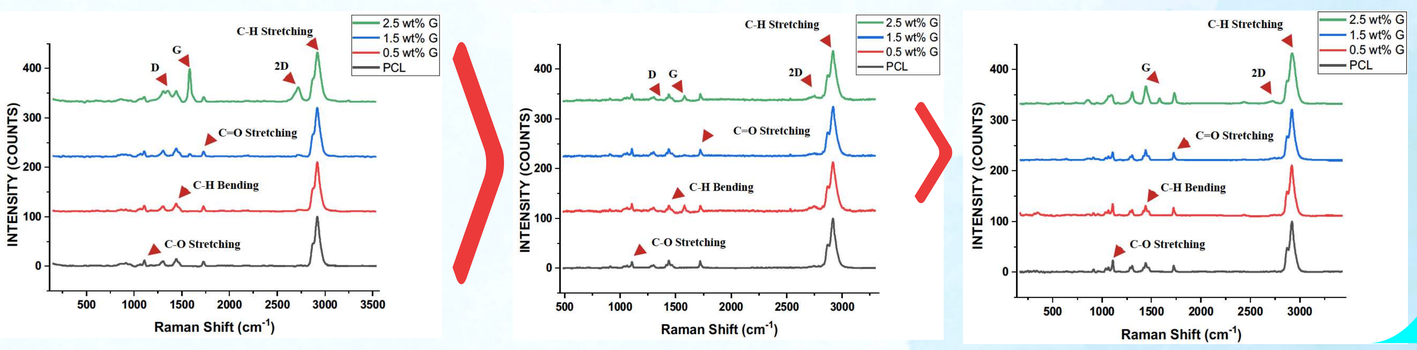
Groups	The shortest Distance (µm)			The longest distance (µm)			Surface area (µm2)			p-value*	
	Hours										
	0	24	48	0	24	48	0	24	48	24	48
PCL	165. 0	53.9	24.1	323.9	323.9	237.7	668.1	587.7	402.4	0.01	0.00
0.5 wt% G		28.2	0		316.0	303.4		482.1	340.6		
1.5 wt% G		236.9	0		191.7	132.7		236.9	106.4		
2.5 wt% G		278.5	0		262.1	81.1		278.6	23.4		

*: There was a significant difference between groups in the area of closure defect on 24 hours ($p<0.05$) and 48 hours ($p<0.001$).

C. BIOCOMPATIBILITY



D. BIODEGRADATION



PCL spectra had three significant absorption peaks. Absorption bands located around 2900 and 2800 cm^{-1} were attributed to asymmetric and symmetric C-H stretching, bands located between 1730 and 1750 cm^{-1} were assigned to C=O stretching, and the band located at 1150 cm^{-1} was linked to the presence of C-O stretching. Following degradation, the intensity of PCL in the spectrum decreased, confirming its degradation. The highest intensity of the change in asymmetric and symmetric C-H stretching occurred at 3 months, while the peak intensity of the change in C=O stretching and C-O stretching occurred at 4 months. Due to the absence of these peaks, which are capable of forming hydrogen bonds with water molecules, the ability to absorb water decreased when the three PCL peaks decreased. This indicated that the capacity to absorb water was reduced.(4)

5. CONCLUSION

A transition occurred from a hydrophobic (PCL) to a hydrophilic surface; 2) a high concentration of graphene had larger pores (2.5 wt% G) than a low concentration and PCL; 3) mechanical properties were enhanced by the addition of graphene; 4) the scaffolds had chemoattractant ad biocompatible with osteoblast-like (MG-63) cells; and 5) these scaffolds with various concentration of graphene had good biodegradation process

6. REFERENCES

[1] A. Sola, J. Bertacchini, D. A'vella, L. Anselmi, T. Maraldi, S. Marmiroli, M. Messori. *Mater Sci Eng*. **2019**, 96, 153.
[2] Y. Tamada, Y. Ikada. *Polymer*. **1993,** 34, 2208.
[3] B. Feng, J, Z. Jinkang, W. Zhen, L. Jianxi, C. Jiang, L. Jian, M. Guolin, D. Xin. *Biomed Mater.* **2011,** 6, 015007.
[4] S. Anitasari. CZ. Wu, YK. Shen, **2023**. *Bioengineering.* 2023,10,305.
[5] O. Guler, N. Bagci. *J Mater Res Tech*. **2020,** 9,6808.